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Predictive Model to Assess Risk for Cardiac Allograft Vasculopathy: An Intravascular Ultrasound Study

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Objectives. **This study was performed to assess the influence and interdependence of immunologic and nonimmunologic risk factors in the development of cardiac allograft vasculopathy. Another primary objective was to establish a clinically useful model for risk assessment of cardiac allograft vasculopathy that would facilitate identifying those heart transplant recipients likely to have severe intimal proliferation and thereby at greatest risk for adverse clinical events.**

Background. **To our knowledge, no comprehensive intravascular ultrasound study has assessed the relative influences of both nonimmunologic and immunologic factors in the development of cardiac allograft vasculopathy, currently the major limitation to long-term cardiac allograft survival.**

Methods. **Using a computer-assisted model of stepwise logistic regression, immunologic and nonimmunologic risk factors were evaluated to help identify the development of severe intimal thickening in 101 subjects who underwent intravascular ultrasound. Prospective validation of the findings was performed in a separate consecutive cohort of 37 heart transplant recipients, and the accuracy of this model to predict a relative risk >1 for the development of severe intimal hyperplasia was assessed.**

Results. **Significant independent predictors of severe intimal hyperplasia in this model included a donor age >35 years, a first-year mean biopsy score >1 (a measure not only of severity of rejection, but also of frequency of insidious rejection) and hypertriglyceridemia at two incremental levels of risk (150 to 250 mg/dl [1.70 to 2.83 mmol/liter] and >250 mg/dl [2.83 mmol/liter]). Based on the absence (0) or presence (1) of these factors, 12 individual categories of risk were ascertained with increasing relative risks and predicted probabilities for severe intimal hyperplasia. Prospective validation of this model revealed a sensitivity and specificity of 70% and 90%, respectively, and the positive and negative predictive values were 85% and 80%, respectively. Additionally, subjects with severe intimal thickening had a fourfold higher cardiac event rate than those without severe intimal proliferation on intravascular ultrasound.**

Conclusions. **This study establishes a clinically useful predictive model that can be applied to individual heart transplant recipients to assess their risk for developing significant cardiac allograft vasculopathy and, thus, aids in the identification of patients at risk for cardiac events in whom closer surveillance and risk factor modification may be warranted.**

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The long-term success of heart transplantation continues to be jeopardized by the development of cardiac allograft vasculopathy, an unusually accelerated form of coronary artery disease responsible for the majority of allograft losses in patients who survive >1 year (1). This disease entity is characterized by rapidly progressive vascular myointimal hyperplasia that eventually leads to coronary lumen compromise and the subsequent catastrophic manifestations of myocardial infarction, allograft dysfunction and sudden death (2,3).

Intravascular ultrasound has been used to identify the presence of myointimal proliferation in heart transplant recipients and has been found to do so in an accurate and reproducible manner. This imaging modality has also been found (4,6) to complement coronary angiography in the morphologic assessment of cardiac allograft vasculopathy because it can identify significant intimal thickening even in the presence of normal findings on the coronary angiogram. Furthermore, intravascular ultrasound not only plays a useful role in the morphologic characterization of cardiac allograft vasculopathy, but it also allows prediction of clinical outcome in heart transplant recipients with significant intimal hyperplasia. A recent study by Mehra et al. (7) demonstrated that in heart transplant recipients, the presence of severe intimal hyperplasia is associated with a higher risk of cardiac events than the presence of mild or moderate intimal proliferation, even in the presence of normal angiographic results.

The development of myointimal hyperplasia in the graft vascular bed requires loss of the protective integrity of endothelium (8). This loss of vasoprotection may arise from the concerted actions of several nonimmunologic risk factors, operating within a milieu conditioned by adverse host immu-

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nologic responses to the foreign cardiac allograft (9,10). This hypothesis has fueled an intense search for the link between several nonimmunologic risk factors, such as abnormal lipid characteristics and obesity (11–13), diabetes mellitus, donor and recipient age and gender (14–16), cold ischemic time and cytomegalovirus infection (17,18) as well as immunologic factors, including cellular or humoral allograft rejection, histocompatibility (19–21) and elevated soluble interleukin-2 receptor levels (22), with the subsequent development of cardiac allograft vasculopathy. Unfortunately, these studies have been limited in examining the relative magnitudes of risk arising from the presence of such risk factors. Although these investigations are of importance in enhancing our understanding of the pathophysiology of cardiac allograft vasculopathy, they do not provide enough information to enable the clinician to assess an individual heart transplant recipient's risk for cardiac allograft vasculopathy.

The purpose of the present investigation was 1) to assess the influence and interdependence of immunologic and nonimmunologic risk factors in the development of severe intimal proliferation; 2) to establish a clinically useful model for risk assessment of cardiac allograft vasculopathy; and 3) to prospectively validate the accuracy of this predictive model in identifying those heart transplant recipients likely to have severe intimal proliferation and thus be at greatest risk for adverse clinical events.

Methods

Patients. Using intravascular ultrasound, we studied a cohort of 138 consecutive and different heart transplant recipients at the time of routine annual coronary angiography between January 1991 and June 1994. All recipients received identical triple-drug immunosuppressive therapy (cyclosporine, prednisone and azathioprine) and were free of acute rejection and infection at the time of the ultrasound study. Patients were excluded from the study if they were ineligible for cardiac catheterization, died during the initial hospital stay or refused to give consent. Written informed consent was obtained from all patients, and the study was approved by the institutional review board.

Nonimmunologic risk factors. Fasting lipid profiles (serum triglyceride, total cholesterol, low density lipoprotein [LDL] and high density lipoprotein [HDL] levels) were assessed in all patients at the time of ultrasound examination. Serum triglyceride levels were evaluated after an overnight 10-h fast and were measured enzymatically using the glycerol phosphate oxidase method (Boehringer Mannheim). Obesity assessment (percent weight gain, body mass index), hypertension (defined according to the Report of the Fifth Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure [JNC V] criteria) (23), diabetes mellitus, donor age and gender, ischemic time, time since transplantation and cytomegalovirus infection (defined as a clinical syndrome in association with either new seroconversion, positive blood cultures or evidence of tissue invasion) requiring therapeutic intervention with intravenous gancyclovir were also assessed in all heart transplant recipients.

Immunologic risk factors including allograft rejection. Rejection surveillance during the first year consisted of weekly endomyocardial biopsies for the first month after transplantation, biweekly until the third month, monthly until the sixth month and bimonthly thereafter until completion of the first year. Thus, the routine protocol mandates the performance of 14 biopsies during the first year. All biopsies were graded on the basis of the standard biopsy grading scheme developed by the International Society for Heart and Lung Transplantation (ISHLT) (24). The criteria for treatment of cellular rejection included the finding of a 3A or higher biopsy grade. If treatment was initiated for a lower biopsy grade, it was done only in the presence of hemodynamic compromise as evidenced by one or more of the following: 20% decrease in ventricular ejection fraction; increase in pulmonary capillary wedge pressure ≥25%; and 25% decrease in cardiac index or an absolute value <2.0 liters/min per m^2. The protocol for treatment of acute cellular rejection in the absence of hemodynamic compromise involved 2 to 5 mg/kg body weight per day of oral corticosteroids or intravenous equivalent for 3 days, then a 5-day taper to baseline. In the event of hemodynamic compromise, 500 to 1,000 mg of intravenous steroids was administered for 3 days, followed by rapid return to baseline. Antilymphocytic antibodies (OKT3) were given only for grade 3B or 4 rejection with hemodynamic compromise. Only two patients in the present study cohort required use of antilymphocytic antibodies (OKT3) for reversal of acute rejection. All episodes of treated rejection mandated the performance of a follow-up biopsy in 1 week for documentation of resolution or evidence for regression to a lower rejection grade. Thus, episodes of treated cellular rejection were analyzed in all study patients.

In addition, a first-year mean biopsy rejection score was determined for all patients. This score was adapted from the standardized ISHLT criteria (24), and individual scores were assigned to each defined biopsy grade as follows: ISHLT grade 0 = 0; grade IA = 1; grade IB = 2; grade 2 = 3; grade 3A = 4; grade 3B = 5; and grade 4 = 6. The mean biopsy score was computed as the average of all biopsy scores during the first year after transplantation.

In addition, episodes of vascular rejection and the number of human leukocyte antigen (HLA)-A, -B or -DR matches between the donor and recipient were assessed in all patients. Acute vascular rejection was defined by the presence of hemodynamic compromise, along with histologic evidence of endothelial cell activation and a positive immunofluorescence on biopsy. More important, detailed immunosuppression regimens were assessed in all patients by calculating the cumulative prednisone dose (total amount of intravenous and oral prednisone consumed [g]), average daily prednisone dose (mg/kg per day), cumulative cyclosporine dose (total amount of intravenous and oral cyclosporine consumed [kg]), mean cyclosporine dose (mg/kg per day) and mean cyclosporine levels (pg/ml) and cumulative (total amount of oral azathio-

prine consumed [kg]) mean azathioprine (mg/kg per day). Use of cytolytic therapy (OKT3 or antithymocyte globulin) and methotrexate was also assessed for each study patient.

Intravascular ultrasound. *Coronary ultrasound procedure.* Coronary ultrasound was performed as previously reported (5,6). After administration of 200 μg of intracoronary nitroglycerin, the ultrasound catheter was positioned in the distal segment of the target vessel over a 0.014-in. (0.036-cm) guide wire. The target vessel was selected by the presence of at least three easily definable and reproducible branch points to assist in the accurate and serial assessment of three regions of interest. The guide wire was removed, and the ultrasound catheter was advanced to the distal end of the ultrasound sheath under fluoroscopic guidance. The drive module was then engaged, and continuous images of the coronary artery were obtained as the ultrasound transducer was slowly withdrawn. Additionally, fluoroscopic pictures and audio annotations were used to ensure the correct localization of the artery segment for subsequent off-line analysis. Guiding catheter pressure, ST segment changes and cardiac rhythm were continuously monitored during the procedure. After ultrasound images were obtained, both the transducer and sheath were removed, and a final angiogram was obtained to confirm the patency of the coronary vessel. No complications were encountered except for coronary artery vasospasm, readily reversed by intracoronary infusion of nitrates or verapamil.

Coronary ultrasound assessment. Intravascular ultrasound measurements were performed by an investigator who had no knowledge of the previous assessment of risk factors. Three coronary sites per vessel (proximal, middle and distal), for a total of 414 coronary artery segments, were evaluated. The left anterior descending coronary artery was examined in 112 patients, circumflex artery in 8 and right coronary artery in the remaining 18 subjects. Maximal intimal thickness and intimal area were obtained by tracing the lumen vessel wall interface and the external border of the intimal layer. Intimal index was calculated as the ratio of intimal area to total vessel area. The intraobserver (4.1%) and interobserver (5.4%) variability was assessed as previously reported (14) and demonstrated good test reproducibility. Lesions were further assessed with regard to plaque location, morphology and eccentricity. The predominant morphologic characteristics of the vessels studied revealed diffuse and concentric lesions in 86%, whereas only 14% of vessels studied revealed proximal, focal and eccentric lesions. Severe intimal thickness was defined on the basis of the scheme previously described by St. Goar et al. (4), wherein severe intimal thickening is represented by >0.5 mm of intimal proliferation involving >180° of the vessel circumference or any intimal layer >1.0 mm in any one area of the vessel circumference. All values less than this threshold were deemed to represent "nonsevere" intimal proliferation. This threshold of severity denotes the "unequivocal" presence of intimal thickening and is beyond the 98th percentile of any published "normal" values. Moreover, we previously demonstrated (7) that severe intimal proliferation by intravascular ultrasound is predictive of cardiac events, even in the absence of angiographic abnormalities.

Cardiac events. Cardiac events analyzed included sudden cardiac death, myocardial infarction and persistent allograft dysfunction, despite augmented immunosuppression, as well as the need for coronary revascularization by percutaneous techniques (angioplasty, atherectomy, stent implantation) or coronary artery bypass surgery.

Statistical methods. Statistical analysis was performed using the BMDP 386 Dynamic statistical software module for stepwise logistic regression (BMDP-LR). Logistic regression analysis works in a similar manner to linear regression. Both methods result in an equation describing a curve of best fit. However, a logit transform is applied so that predicted probabilities from the equation will not exceed the boundaries of 0 and 1. The resulting curve is sigmoidal, with asymptotes at 0 and 1, rather than a line. A goodness-of-fit test is used to determine whether the model describes the data well rather than the correlation coefficient in linear regression. The stepwise fitting of the model excludes variables with coefficients not statistically different from zero.

The starting set of the previously described immunologic and nonimmunologic variables was selected, and the influence of these variables on the development of severe intimal hyperplasia by intravascular ultrasound was then assessed. The variables for entry into or removal from the computer-assisted model were p values of the maximal likelihood ratio of 0.10 and 0.15, respectively. The improvement chi-square (null hypothesis: "there is no improvement") and goodness-of-fit chi-square tests (null hypothesis: "the model fits the data") were considered significant at $p < 0.10$ and $p > 0.20$, respectively. Interaction terms were not allowed to remain in the model unless the component main effects were considered significant.

Normally distributed data are reported as mean value ± SD. Differences between categoric variables were assessed using the chi-square or the Fisher exact test. An unpaired Student *t* test was used to define differences between continuous variables in subgroups. Kaplan-Meier actuarial analysis was used to assess event-free survival for the study cohort.

Results

Clinical characteristics. The present study group included 138 consecutive and different heart transplant recipients (116 men, 22 women; mean [±SD] age 50 ± 11 years, range 22 to 68) who were studied a mean of 2.3 ± 1.4 years after transplantation (range 1 to 4). The first 101 consecutive patients formed the "derivation" cohort and were utilized to determine the predictive variables that could assess a subject's risk for having severe intimal proliferation, whereas the next 37 consecutive patients in the "validation" cohort were prospectively utilized to assess the clinical accuracy of these predictive factors. Table 1 presents comparative patient characteristics of the derivation and validation cohorts with regard to nonimmunologic (cold ischemic time, lipid characteristics, weight gain,

Table 1. Patient Characteristics

	Derivation Cohort (n = 101)	Validation Cohort (n = 37)
Age (yr)	50 ± 10.5	52 ± 12
Gender (M/F)	86/15	30/7
Time after transplantation (yr)	2.3 ± 1.4	2.2 ± 1.7
Intimal thickness (mm)	0.49 ± 0.37	0.50 ± 0.38
Intimal index	0.22 ± 0.16	0.18 ± 0.11
Ischemic time (min)	181 ± 58	194 ± 66
Total cholesterol (mg/dl)	239 ± 62	249 ± 69
LDL cholesterol (mg/dl)	151 ± 50	143 ± 35
HDL cholesterol (mg/dl)	46 ± 15	50 ± 12
Triglycerides (mg/dl)	198 ± 97	231 ± 164
Weight gain (%)	12.3 ± 11	11.9 ± 9
Donor age (yr)	25 ± 9	27 ± 11
Donor gender (M/F)	67/34	21/16
Cytomegalovirus infections (%)	16	14
Diabetes mellitus (%)	36	32
HLA matches	0.8 ± 0.9	0.9 ± 0.6
Treated cellular rejections (%)	39	33
Biopsy rejection score	0.99 ± 0.39	0.94 ± 0.40

Data presented are mean value ± SD or number or percent of patients. F = female; HDL = high density lipoprotein; HLA = human leukocyte antigen; LDL = low density lipoprotein; M = male.

cytomegalovirus infections, diabetes mellitus, donor age, donor gender) and immunologic (episodes of allograft rejection, HLA matches, mean first-year biopsy rejection score) risk factors. Thus, the derivation and validation groups were similar with regard to the prevalence of all risk factors studied.

Statistical model. Of all immunologic and nonimmunologic variables assembled, the statistical method of stepwise logistic regression screened out those that did not meet statistical significance. This method allowed for evaluation and control of confounding or interacting variables. In the final model, donor age >35 years, serum triglyceride levels of 150 to 250 mg/dl (1.70 to 2.83 mmol/liter) (level 1) and those >250 mg/dl (2.83 mmol/liter) (level 2), as well as a first-year mean biopsy rejection score emerged as significant independent predictors of severe intimal thickening. Variables that were not found to be significant included recipient age and gender, donor gender, cold ischemic time, serum cholesterol, LDL cholesterol, HDL cholesterol, weight gain after transplant, time elapsed since transplantation, histocompatibility, acute vascular rejection and cytomegalovirus infection.

Donor age and hyperlipidemia. Donor age was grouped around the cutpoint of 35 years of age in accordance with the central tendency of our data and previous findings (25). Serum triglyceride levels were classified into three groups: 1) <150 mg/dl (1.70 mmol/liter); 2) 150 to 250 mg/dl (1.70 to 2.83 mmol/liter) (level 1); and 3) >250 mg/dl (2.83 mmol/liter) (level 2). These cutpoints were based on the findings of the Framingham Heart Study (26), which established an independent and incremental risk for the development of coronary artery disease around the two distinct levels of 150 to 250 mg/dl.

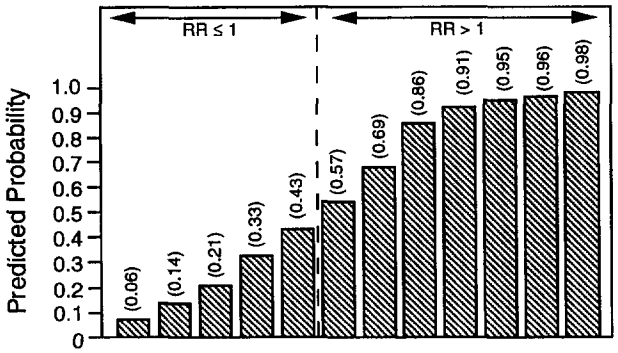
Cellular allograft rejection and mean biopsy score. There was no relation between episodes of treated cellular allograft rejection and the presence of severe intimal proliferation. Conversely, the first-year mean biopsy score, which approximated a gaussian distribution with a median around 1, was found to be a significant independent predictor of severe intimal hyperplasia. To further examine the clinical value of the mean biopsy score, we assessed the relation of this score with the number of biopsy procedures, as well as with the number of treated episodes of rejection. Heart transplant recipients with a mean biopsy score of >1 had a greater number of biopsies performed than patients with a biopsy score ≤1 (16 ± 4.8 vs. 13 ± 2.6, p = 0.02). In addition, a positive correlation between the number of biopsies performed and mean biopsy score (r = 0.36, p = 0.002) was found, suggesting that most additional biopsies were performed in the presence of higher rejection grades. Furthermore, patients with treated episodes of rejection had higher mean biopsy rejection scores than patients without episodes of rejection (1.22 ± 0.37 vs. 0.89 ± 0.39, p = 0.003). When episodes of cellular rejection were treated with augmented immunosuppression, the mean number of additional biopsies performed in the ensuing 4 weeks was 1.3 ± 0.4.

Immunosuppression. There were no significant differences between immunosuppression regimens in patients with severe and nonsevere intimal thickness. Average daily cyclosporine dose (3.8 vs. 3.9 mg/kg per day, p = 0.9), mean cyclosporine blood levels (168 vs. 164 pg/ml, p = 0.4), cumulative cyclosporine dose (0.37 ± 0.22 vs. 0.45 ± 0.28 kg, p = 0.13), cumulative prednisone consumption (12.0 vs. 13.8 g, p = 0.14), average prednisone dose (0.11 vs. 0.10 mg/kg per day, p = 0.3) and cumulative azathioprine (0.11 ± 0.08 vs. 0.16 ± 0.12 kg, p = 0.02) did not differ significantly between patients with nonsevere and severe intimal proliferation, respectively. Linear regression analysis of the interaction of cumulative prednisone dose used for treatment of acute rejection episodes with the first-year mean biopsy rejection score revealed no significant correlation (r = 0.11, p = 0.3).

Analysis of predictive variables. On the basis of the results of statistical analysis, the significant variables that emerged as independent predictors were donor age >35 years; serum triglyceride levels 150 to 250 mg/dl (1.70 to 2.83 mmol/liter) (level 1) and >250 mg/dl (2.83 mmol/liter) (level 2); and a first-year mean biopsy rejection score >1. The final model can be shown as an equation of predicted log odds as follows:

$$\text{Log odds} = -2.8 + 3.5\,(\text{Donor age} > 35\text{ years}) + 1.5\,(150\text{ to }250\text{ mg/dl}) + 2.1\,(\text{Triglycerides} > 250\text{ mg/dl}) + 1\,(\text{First-year mean biopsy score} > 1).$$

This equation emphasizes the relative contributions of these significant variables in the development of severe intimal thickening. The predicted log odds can then be converted to relative risk and predicted probability ($\hat{p}$), both reflecting the chance that a given patient will have severe intimal hyperplasia, by the formula:



Risk Category:	1	2	3	4	5	6	7	8	9	10	11	12
Donor Age > 35 yrs	0	0	0	0	0	0	1	1	1	1	1	1
Triglycerides 150-250 mg/dl	0	0	1	0	1	0	0	0	1	0	1	0
Triglycerides > 250 mg/dl	0	0	0	1	0	1	0	0	0	1	0	1
Mean First Year Biopsy Score >1	0	1	0	0	1	1	0	1	0	0	1	1

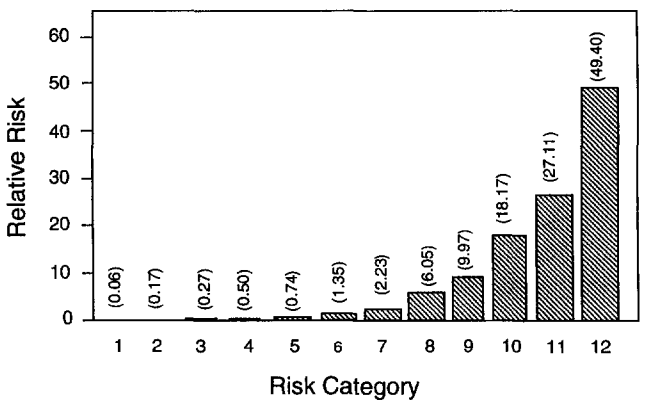
Figure 1. Predicted probability for severe intimal hyperplasia by individual patient risk categories. Categories 1 to 5 define relative risk (RR) ≤1; whereas categories 6 to 12 define RR >1 for cardiac allograft recipients with versus without the adverse risk profile (conversion factor for serum triglyceride levels from mg/dl to mmol/liter is 0.0113).

$$\text{Relative risk} = e^{(\hat{p}/1-\hat{p})} \quad \text{and} \quad (\hat{p}) = e^{(\hat{p}/1-\hat{p})}/1 + e^{(\hat{p}/1-\hat{p})}.$$

Based on the absence (coded as 0) or presence (coded as 1) of these predictive factors, 12 possible categories of patient profiles with increasing probabilities for having severe intimal proliferation can be established and are delineated in Figure 1. Furthermore, relative risks for each patient category can also be calculated and are shown in Figure 2.

Clinical use of predictive variables. The prediction of probability of having severe intimal thickening in any given patient requires the categorization of individual patients into one of the 12 possible groups (Fig. 1 and 2). Thus, if the clinician wishes to assess the risk for development of severe intimal hyperplasia in a 52-year-old heart transplant recipient of a 16-year-old allograft donor with a serum triglyceride level of 112 mg/dl (1.27 mmol/liter) and a first-year mean biopsy rejection score of 0.94, this particular patient would fit into category 1 (0, 0, 0, 0) and have a predicted probability of 0.06 for developing severe intimal hyperplasia. Conversely, a patient with a 47-year-old cardiac donor allograft, a serum triglyceride level of 278 mg/dl (3.14 mmol/liter) and a biopsy rejection score of 1.3 would be in category 12 (1, 0, 1, 1) and have a predicted probability of 0.98 for developing significant intimal thickness.

Figure 2. Relative risk (RR) for individual patient categories for development of severe intimal hyperplasia classified by risk factor profile into 12 patient categories. Categories 1 to 5 denote RR <1, whereas categories 6 to 12 denote RR >1.



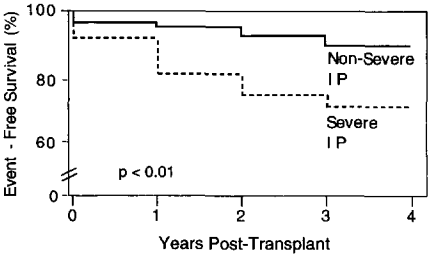


Figure 3. Actuarial probability of cardiac event-free survival for severe versus nonsevere intimal proliferation (IP).

Prospective validation. A cohort of 37 consecutive heart transplant recipients was used to define prospectively the accuracy of this model for predicting a relative risk >1 of having severe intimal hyperplasia. Using the predictive model, Categories 1 to 5 were defined to denote a relative risk ≤1, whereas Categories 6 to 12 were associated with a relative risk >1 (Fig. 1). Sixteen patients were found to have severe intimal hyperplasia, whereas the remaining 21 patients had minimal or mild intimal thickening. Of this group, use of the predictive model accurately classified 19 to 21 patients as having a relative risk of ≤1, and 11 of 16 patients were correctly classified with a relative risk >1. Thus, the sensitivity and specificity were 70% and 90%, respectively, whereas the positive and negative predictive values were 85% and 80%, respectively. The overall accuracy of this model was 81%.

Morphologic characterization and cardiac event analysis. A total of 25 cardiac events occurred in the entire study group. Of these, there were 14 sudden deaths, 6 myocardial infarctions and persistent allograft dysfunction despite augmented immunosuppression and 5 coronary revascularizations (1 angioplasty, 2 directional atherectomies, 2 stent implantations), yielding an event rate of 7.8%/patient per year. In addition, patients with severe intimal proliferation were three times more likely to have a cardiac event than patients with nonsevere intimal thickening (30% vs. 10.2%, p = 0.009). Actuarial probability of event-free survival in heart transplant recipients with severe intimal proliferation compared with nonsevere intimal thickening is depicted in Figure 3. The morphologic assessment of the distribution of intimal proliferation revealed that 86% of the lesions were concentric (>180°) and 81% diffuse, whereas 14% were eccentric and 19% focal. Because donor age >35 years emerged as one of the most important

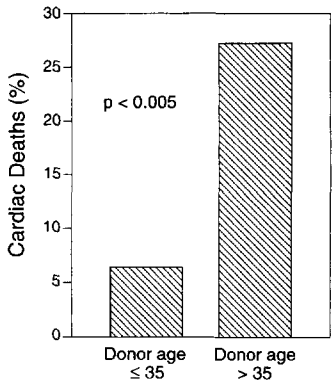


Figure 4. Cardiac death rates in subjects with severe intimal proliferation and a donor age >35 years versus those with donor age ≤35 years. Recipients of a donor allograft >35 years old have a fourfold higher incidence of cardiac death.

predictive factors for having severe intimal proliferation, we also examined the prevalence of cardiac events in the group of patients with severe intimal proliferation and donor age >35 years compared with patients with a donor age ≤35 years. The incidence of cardiac death was four times higher in the patients with severe intimal proliferation and donor age >35 years than in those with a donor age ≤35 years (27% vs. 6%, $p < 0.005$) (Fig. 4). Similar differences were evident in the patients with advanced donor age when all cardiac events were assessed (42% vs. 14%, $p < 0.001$). Of particular note is the finding that 88% of the lesions in this high donor age group were diffuse and concentric, whereas only 12% demonstrated focal and eccentric intimal thickening.

Angiographic data in the patient cohort with severe intimal proliferation revealed that 23% of the subjects demonstrated normal angiographic findings despite intravascular ultrasound evidence of disease. More significantly, 32% of the study cohort that had a cardiac event had no antecedent angiographic abnormality despite demonstration of severe intimal proliferation by intravascular ultrasound.

Discussion

Predictive model. According to our risk assessment model, the results of the present intravascular ultrasound study indicate that the presence of donor age >35 years, hypertriglyceridemia and a high first-year mean biopsy rejection score are independently and significantly associated with the risk of having severe intimal thickening. This predictive model not only elucidates the relation of these three immunologic and nonimmunologic risk factors among each other, but also allows identification of heart transplant recipients at risk for the deleterious consequences of cardiac allograft vasculopathy. Moreover, prospective validation of this model determined that use of these predictive factors provides a highly accurate assessment of risk for having severe intimal proliferation, with positive and negative predictive values of 85% and 80%, respectively.

Donor age and cardiac allograft vasculopathy. The findings of the present study are consistent with the notion that an interplay among an aged allograft, hyperlipidemia and insidious immunologic damage from allograft rejection may be associated with the development of significant intimal hyperplasia (25). A critical assessment of the relative influences of these risk factors within the model suggests that advanced donor age accounts for one of the highest risks for developing severe intimal hyperplasia. Analysis of donor age in the context of the predictive model suggests that if a heart transplant recipient were to have a donor age >35 years as the sole risk factor, with normal serum lipid levels and a first-year biopsy rejection score <1, that patient would have a predicted probability of 0.69 and a relative risk of 2.23 for having severe intimal thickening. In addition, the presence of donor age >35 years confers a fourfold higher risk of cardiac death than cardiac events in recipients of younger (<35 years) allografts. Recent studies that have evaluated the influence of donor age in the development of cardiac allograft vasculopathy have yielded similar results. Tuzcu et al. (27) examined 22 heart transplant recipients serially at baseline and 1 year after transplantation using intravascular ultrasound and demonstrated that patients with a donor age >35 years developed the greatest increase in intimal proliferation. Another recent investigation by Gao et al. (28) revealed that recipients of donor hearts with advanced donor age (>40 years) were less likely to be free from development of cardiac allograft vasculopathy than those who received younger allografts (<40 years). The reasons for this influence of donor age on the development of intimal hyperplasia remain speculative. However, it is likely that the older allograft may be more susceptible to endothelial damage resulting from the host–graft immunologic and nonimmunologic interaction.

Hyperlipidemia and cardiac allograft vasculopathy. The role of hyperlipidemia and its influence on cardiac allograft vasculopathy has been a matter of intense scrutiny. Several studies (11,29–32) have uniformly demonstrated that hypercholesterolemia, increased LDL cholesterol and hypertriglyceridemia begin to develop 1 year after transplantation. Although the exact cause of posttransplantation hyperlipidemia remains uncertain, obesity and immunosuppressive agents are thought to play an integral role in its development. It has been demonstrated (33,34) that cyclosporine decreases lipoprotein lipase activity and inhibits prednisone clearance by its interaction with the hepatic cytochrome P-450 system. Furthermore, prednisone may increase hepatic apolipoprotein B production (35). These effects result in impaired very low density lipoprotein (VLDL) and LDL clearance and lead to hypercholesterolemia and hypertriglyceridemia. An intriguing new concept relates to the role of insulin resistance in the development of hypertriglyceridemia and its consequent propagation of cardiac allograft vasculopathy. However, confirmation of this concept awaits further scrutiny. The findings of the present study not only suggest the predictive value of elevated serum triglyceride levels in the development of severe intimal hyperplasia, but also suggest that this risk is incremental and

increases exponentially with the degree of hypertriglyceridemia. The mechanisms underlying the influence of elevated triglyceride levels on vascular intimal thickening in heart transplant recipients may be diverse and can be related either directly to the atherogenic potential of VLDL particles or may result from the metabolic consequences of hypertriglyceridemia that include an increase in small, dense LDL particles and low levels of HDL cholesterol (36).

Cellular rejection and cardiac allograft vasculopathy. One issue that has remained a matter of controversy is the role of cellular allograft rejection in the development of cardiac allograft vasculopathy. Although several studies exist that dispute an association between the two, some studies have linked acute cellular allograft rejection with cardiac allograft vasculopathy (1,19). Uretsky et al. (1) demonstrated that there is an association between the occurrence of two or more major rejection episodes and the prevalence and severity of cardiac allograft vasculopathy, thus suggesting that multiple immunologic events may be necessary for the development of cardiac allograft vasculopathy. In a separate study that analyzed the association between cardiac allograft vasculopathy and both frequency and histologic severity of rejection, Schütz et al. (37) demonstrated higher rejection scores in patients with than without manifestations of cardiac allograft vasculopathy. In a study of failed human allografts, Winters et al. (12) suggested that even minimal endomyocardial rejection may contribute insidiously to the development of cardiac allograft vasculopathy. These varied observations suggest that not only is the number of episodes of rejection important, but the degree of histologic severity, particularly low grade insidious cellular rejection, may also play a crucial role in the development of cardiac allograft vasculopathy. Although episodes of treated cellular rejection were not an independent risk factor for severe intimal proliferation in our investigation, the mean first-year biopsy rejection score emerged as a significant risk factor. The clinical value of the average biopsy rejection score lies in its ability to account not only for the frequency of high grade rejection occurrence, but also the risk conferred by lower grades of rejection severity that would otherwise not be construed to represent clinical rejection. Furthermore, the presence of a mean first-year biopsy rejection score >1, in conjunction with a donor age >35 years and the presence of hypertriglyceridemia, adds to the risk for development of severe intimal thickening. As our model demonstrates, when a biopsy score >1 accompanies a donor age >35 years as a risk factor, the predicted probability for severe intimal thickness increases from 0.69 to 0.86 in any individual heart transplant recipient, and the addition of hypertriglyceridemia enhances this predicted probability to >0.90.

Clinical significance. The clinical ramifications of the present study lie in the recognition of the heart transplant recipient most likely to suffer the devastating consequences of cardiac allograft vasculopathy. Not only does our predictive model allow close scrutiny of the individual influences of donor age, hypertriglyceridemia and allograft rejection as well as their interrelation in the development of severe intimal thickening in an individual heart transplant recipient, but it does so with an overall accuracy of 81%. Indeed, the group of subjects identified by this model are also those most likely to suffer a cardiac event, as was evident by the threefold higher cardiac event rate in the heart transplant recipients with severe intimal proliferation. Thus, it may seem reasonable to suggest that in this highly select group, closer monitoring for rejection and modification of hypertriglyceridemia by control of predisposing factors may be warranted.

Study limitations. Certain potential limitations of this study merit emphasis.

1. An important issue relates to the lack of baseline intravascular ultrasound studies that would have allowed exclusion of donor-transmitted disease as a potential confounding variable. A recent intravascular ultrasound investigation of advanced donor age allografts (38) has alluded to the significant transmission of "occult" coronary disease at the time of transplantation. This finding is particularly important to our study because advanced donor age was a very potent risk factor for severe intimal proliferation. Evidence to suggest that these study results are less likely to have arisen solely due to this phenomenon are as follows: a) The prevalence of severe intimal proliferation in our advanced donor age group (>35 years) is 94%, which is higher than that of donor-transmitted disease; b) the disease distribution was more diffuse and concentric, as opposed to the predominance of proximal, focal and eccentric intimal thickening in donor-transmitted disease; c) more importantly, heart transplant recipients with the higher donor age (>35 years) were four times more likely to suffer a cardiac death or other cardiac-related event as a result of cardiac allograft vasculopathy. These results emphasize that the relation of advanced donor age with severe intimal proliferation is unlikely to have resulted from simply the effect of disease transmitted at the time of transplantation. Furthermore, other recent investigations (27,28) have also suggested an important role for advanced donor age in determining progression of intimal proliferation.

2. The possible beneficial influence of pharmacologic agents, such as the calcium entry blocking agent diltiazem (39), as well as lipid-lowering therapy on the development of cardiac allograft vasculopathy is a potential limitation. However, only 11 heart transplant recipients were receiving diltiazem and 19 lipid-lowering medications during the study period and are therefore unlikely to have affected the results significantly.

3. Another important limitation relates to our use of a first-year mean biopsy rejection score as a marker for continued immunologic stimulation of the transplanted allograft. Although this system is useful in assessing the graded severity, frequency and influence of insidious immunologic activation, it does not assess the relation between episodes of rejection occurring after the first year, albeit infrequent, and the development of cardiac allograft vasculopathy.

4. A possible limitation relates to the three-site image analysis intravascular imaging protocol used in this study. More recently, morphometric analysis of intravascular ultrasound obtained after analyzing 10 random sites has been

utilized in an effort to better quantitate intimal proliferation because the routine three-site image analysis has been found to be somewhat less accurate in assessing heterogeneous disease (40).

Conclusions. The development of myointimal hyperplasia, detected accurately by intravascular ultrasound in heart transplant recipients, is influenced by the interdependence of donor age, lipids (serum triglycerides) and allograft rejection. The present study establishes an accurate and clinically useful predictive model that can be applied to individual heart transplant recipients to assess their risk for developing severe intimal proliferation and, thereby, cardiac allograft vasculopathy. Furthermore, this model enables the clinician to identify those heart transplant recipients at high risk for a cardiac event in whom closer monitoring for rejection and risk factor modification may be warranted.

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